

conditions: After the first cycle comprising a denaturing step holding a temperature at 94 °C for 1 minute, and an annealing step and extension step with a DNA polymerase holding a temperature at 65 °C for 4 minutes is performed once, the second cycle comprising a denaturing step holding a temperature at 94 °C for 30 seconds, and an annealing step and extension step with a DNA polymerase holding a temperature at 65 °C for 4 minutes is performed 15 times. After completion of the polymerase chain reaction, the DNA fragment amplified in the polymerase chain reaction is purified by filtering the reaction solution with a spin column (MicroSpin S-400HR manufactured by Pharmacia Biotech). After the end of this DNA fragment is made blunt with a DNA blunting kit (manufactured by TAKARA SHUZO CO., LTD), a phosphate group is added to the 5' end with T4 polynucleotide kinase (manufactured by TAKARA SHUZO CO., LTD).--

IN THE CLAIMS

Please amend the following claims:

28. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into

protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell;

(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indicative of an inhibition of protoporphyrinogen oxidase activity by said test compound.

29. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, and a terminator functional in the host cell;

(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indication of an inhibition of PPO activity by said test compound.

30. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, wherein said promoter is inducible, and a second vector comprising a second DNA fragment which is a DNA capable of inducing the promoter of the first DNA fragment, and a promoter, wherein said promoter is not induced by the second DNA fragment but is functional in the host cell, are operatively linked;

(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indication of an inhibition of PPO activity by said test compound.

31. (Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

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cont.

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen activity, said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, and a terminator functional in the host cell, wherein said promoter is inducible, and a second vector comprising a second DNA fragment in which a DNA being capable of inducing the promoter of the first DNA fragment, a promoter, wherein said promoter is not induced by the DNA fragment but is functional in

the host cell, and a terminator functionable in the host cell are operatively linked;

(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indication of an inhibition of PPO activity by said test compound.

33. (Amended) The method according to any one of claims 28 to 30, wherein the protoporphyrinogen oxidase DNA is a protoporphyrinogen oxidase DNA derived from a group consisting of Dicotyledonous plants, Monocotyledonous plants, algae, mammals, fish, and insects.

Please add the following claims:

-35. The method according to any one of claims 28 to 30, wherein the protoporphyrinogen oxidase DNA is a protoporphyrinogen oxidase DNA derived from a group consisting of Arabidopsis, soybean, oil seed rape, sugar beet, potato, tobacco, corn, rice, wheat, barley, oat, rye, sugar cane, sorghum,

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*D3 cont'd*  
*Chlamydomonas reinhardtii*, *Chlorella*, mouse, rat, human, trout,  
bluegill, carp, cyprinodont, ~~guppy~~, zebra fish, fathead minnow,  
fly, mosquito, cockroach, ~~greasy~~ grind, dragonfly and silkworm  
moth.--

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